Summary

The major objective of this work has been to evaluate *Pichia pastoris* as a host to express the E proteins of dengue viruses. This work has focused on DEN-2 virus as it is the most virulent of all four serotypes and is more prevalent in our country. A carboxy-terminally truncated version of the envelope protein of DEN-2 virus (Den2E protein) was expressed either by itself or in association with the hepatitis B surface antigen (HBsAg) as a fusion partner. Both these proteins were expressed in *P. pastoris* and purified to near homogeneity. The purified recombinant proteins were investigated by gel filtration, sedimentation analysis and electron microscopy. Finally, the immune response they elicit in experimental animals was investigated by a variety of criteria including ELISA, immunofluorescence, immunoprecipitation and virus neutralization assays. The salient features are summarized below:

- This work has demonstrated the expression of a novel chimeric protein, in *P. pastoris*, derived by in frame fusion of the first 395 aa residues of Den2E with the 226 aa residues of HBsAg. The recombinant fusion protein, Den2E-HBsAg was immunopurified to near homogeneity, using the HBsAg component as an affinity handle.

- The second version of Den2E protein (lacking HBsAg fusion partner), was engineered to contain a 6X-His tag at its N-terminus, to facilitate single-step purification by Ni-NTA affinity chromatography.

- Multiple lines of indirect evidence suggested that both the recombinant proteins form high molecular weight aggregates. However, direct electron microscopic visualization showed that only Den2E-HBsAg hybrid protein exists as true VLPs. These hybrid VLPs were recognized by two different neutralizing antibodies (anti-Den2E and anti-HBsAg mAbs) in two different assays (sandwich ELISA and immunoelectron microscopy).

- This is the first report to demonstrate the generation of Den2E containing VLPs in *P. pastoris* without the aid of any other dengue viral structural proteins, known to be essential for virion morphogenesis.

- The data from antibody titration experiments, IFA studies and PRNT assay show that the anti-Den2E-HBsAg antibodies can recognize and neutralize DEN-2 virus more efficiently than antibodies elicited by recombinant Den2E alone.

- A significant finding is that these hybrid particles can function as bivalent immunogens capable of eliciting immune responses against both Den2E as well as HBsAg. The data presented in this thesis suggest that the approach followed to develop the bivalent immunogen has important implications for multivalent vaccine development.